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A gel-based reference map of the porcine hepatocyte proteome☆

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Abstract

The overall goal of our research is to characterize and identify gene expression profiles of porcine hepatic cells. In this study, we have prepared two-dimensional electrophoresis maps of cytosol and membrane fractions from freshly prepared hepatocytes which were pooled from three crossbred pigs (35–69 kg). Following isoelectric focusing with three pH range immobilized pH gradient strips (pH 3–6, 5–8 and 7–10) and staining the second dimension gels with colloidal Coomassie blue, 728 protein spots were picked and digested with trypsin. Extracted tryptic peptides were initially subjected to matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI–TOF-MS) analysis for identification of proteins by peptide mass fingerprinting (PMF). Proteins which were not identified by PMF were analyzed by liquid chromatography–tandem MS. Utilizing publicly available databases [NCBInr, Swiss Prot and expressed sequence tags (EST)], 648 proteins were identified. Of those, 282 were unique proteins and greater than 90% of proteins spots contained single proteins. These data represent the first comprehensive proteomic analysis of porcine hepatocytes and will provide a database for future investigations of endocrine regulation of gene expression and metabolic processes in vitro.
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Keywords: Pig hepatocytes; 2D-PAGE; MALDI–TOF–MS; LCMS/MS

1. Introduction

There is growing interest in porcine hepatocyte models for use in the investigation of swine metabolic processes [1–4], as well as, in human clinical applications for transplantation/artificial liver support [5] or toxicological analyses [6]. In all cases, whether fresh or cultured cells are utilized, an understanding of gene expression at both the mRNA level and at the protein level will improve our understanding of regulatory processes and hepatic function. Indeed, a proteomic approach has been successfully used to assess the effects of physical manipulation and cell harvesting techniques on distribution and/or loss of proteins in rat hepatocytes [7]. In our laboratory, both short-term hepatocyte cultures on collagen [8,9] and long-term (weeks to months) cultures of adult hepatocytes (unpublished) and hepatic stem cells [10] are routinely employed and protein expression profiles during these culture periods are critical to interpret hormonal effects on differentiated cell function. Thus, our aim was to prepare a comprehensive database of the expressed proteins present in freshly prepared hepatocytes to understand potential changes due to culture. Our initial approach is presented here, where

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basic membrane and cytosol fractions were prepared and proteins were separated by two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) and followed by identification with mass spectrometry to catalog the major proteins present in isolated hepatocytes. Our goal was to separate analytical quantities of proteins to yield single discrete protein spots for future comparisons using quantitative 2D-PAGE techniques. Using this approach we have successfully identified over 600 proteins. In addition, by using the apparent molecular weight of separated proteins, a more accurate interpretation of MS spectra and a high level of confidence in assigning protein identifications can be achieved, compared to non-gel based techniques.

2. Materials and methods

2.1. Reagents

Chemicals for electrophoresis including acrylamide, bis-acrylamide, *N*,*N*,*N*′,*N*′-tetra-methyl-ethylenediamine (TEMED), ammonium persulfate, agarose, (3-[3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS), Triton-X 100, ampholytes (Bio-Lytes, pH 3–10), Bromophenol blue (BPB), iodoacetamide, and sodium dodecyl sulfate (SDS) were purchased from Bio-Rad (Hercules, CA). Trizma-Base (Tris), ethylenediaminetetraacetic acid (EDTA), diethyldithicarbamate (DEDTC), dithiothreitol (DTT), octyl glucoside, glycerol, glycine, ammonium sulfate, Coomassie blue G-250, thiourea, and trichloroacetic acid (TCA) were purchased from Sigma (St. Louis, MO). Urea was from Pierce (Sequanal grade, Rockford IL). Chemicals used during sample prep for MS analysis including acetonitrile (ACN) and ammonium bicarbonate were from Sigma. Methanol, chloroform and acetic acid were from Fisher (Suwanee, GA), and trifluoroacetic acid (TFA) was from J.T. Baker (Phillipsburg, NJ). All other chemicals were reagent or HPLC grade.

2.2. Hepatocyte preparation

Care and treatment of all pigs in this study were approved by the Institutional Animal Care and Use Committee of the U.S. Department of Agriculture. Crossbred barrows (Landrace × York × Poland China, $n = 3$, 52 ± 10 kg) were fully fed a corn-soy diet prior to stunning by electric shock and exsanguination. Livers were immediately excised and the left lateral lobe was removed. Hepatocytes were isolated by a two-step collagenase digestion procedure essentially as previously described [9,11], where only a small portion (approximately 80 g) of the lateral lobe was used. Viability of hepatocytes isolated by low speed centrifugation was $89 \pm 2.5\%$ by Trypan blue dye exclusion and aliquots were frozen at −80 °C until used. Cytosol and membrane fractions were prepared by centrifugation and differential solubilization in octylglucoside as described by Ruiz-Cortés et al. [12]. Purified hepatocytes from the three pigs were thawed on ice, pooled, homogenized by brief exposure to ultrasound in buffer [2 mM octylglucoside, 1 mM DEDTC, 10 mM EDTA in 50 mM Tris, pH 8] and centrifuged at 30,000 *g* for 60 min at 4 °C. The supernatant (cytosol) was harvested and the particulate fraction was solubilized by ultrasound in 20 mM Tris containing 50 mM EDTA, 0.1 mM DEDTC and 32 mM octylglucoside, and centrifuged at 16,000 *g* for 15 min at 4 °C. The insoluble pellet was discarded and the supernatant was aspirated and considered to be the membrane fraction. All buffers contained standard mammalian protease inhibitor cocktail (1 μl/ml, P-8340, Sigma). Cytosol and membrane fractions were precipitated by addition of 8–10 volumes of cold chloroform:methanol (4:1), vortexed, sonicated and incubated on ice for 10 min. The solution was centrifuged at 3000 *g* for 10 min at 4 °C. Supernatant solution was discarded and methanol (5 ml) was added to the pellet which was sonicated and centrifuged as before. The final pellet was vacuum dried to remove residual methanol and re-suspended in re-hydration buffer (RHB): 8M urea, 1 M thiourea, 50 mM DTT, 4% CHAPS, 0.5% Bio-Lytes (pH 3–10), and 0.001% BPB, using mechanical disruption and sonication. Insoluble material was removed by centrifugation at 15,000 *g* for 2 min at room temperature and cell material was stored at −80 °C. Concentration of protein for all samples was determined by the method of Lowry following precipitation in 7% TCA and 0.07% Triton-X 100 [13]. BSA was used as a standard and RHB buffer was used as background.

2.3. Two-dimensional polyacrylamide gel electrophoresis

The first dimension isoelectric focusing (IEF) was performed using 11 cm immobilized pH gradient (IPG) gel strips (pH 3–6, 5–8 or 7–10, Bio-Rad) in the IPGphor II system (GE Healthcare, Piscataway, NJ). In-gel re-hydration of pH 5–8 IPG strips was performed in the presence of 200 μg protein in a total volume 200 μl RHB; additional RHB was prepared fresh as needed to dilute the protein sample. The strips were passively re-hydrated for 1 h at 20 °C, followed by active re-hydration at 50 V for 12–15 h and settings for protein focusing were: 500 V for 1 h, then elevated to 8000 V maximum to a total of 46 kVh. The focused strips were rinsed in dH_20 and stored at −80 °C. For the pH 3–6 and 7–10 strips, overnight passive re-hydration was performed with 200 μl RHB alone. Protein (400–500 μg in 400 μl RHB) was loaded at the anodic electrode via a paper bridge using the IPGphor II manifold system (GE Healthcare), according to the manufacturer's instructions. In addition to the dH_20 wick, 300 μl of RHB containing 3.5% DDT was loaded onto a pad which was placed upon the cathode end of each pH 7–10 IPG strip as suggested by Hoving et al. [14]. Isoelectric focusing was performed by ramping the voltage to a maximum of 6000 V and terminated at 20,000 Vh. Focused strips were rinsed in dH_20 and stored at −80 °C.

For the second dimension electrophoresis, the IPG gel strips were incubated with equilibration buffer (0.4M Tris–HCl pH 8.8, 6 M urea, 20% glycerol, 2% SDS, 1% DTT, 0.001% BPB) for 5 min; it was removed and fresh buffer was added for an additional 15 min. Strips were then incubated in the same equilibration buffer, without DTT, but containing 2.5% iodoacetamide for an additional 15 min. Finally, excess iodoacetamide was removed by soaking strips (5 min) in buffer containing equilibration buffer with 1% DTT. Each strip was rinsed in dH_20 and subsequently placed onto a 12% polyacrylamide gel (16 cm × 20 cm) and run with a Tris–glycine buffer system as described by Laemmli [15]. Strips were overlayed with 2.5% low melting agarose sealing solution (0.375 M Tris base, 0.1% SDS, 0.01 M DTT and 0.001% BPB, pH 8.9). Electrophoresis was performed, using the PROTEAN II xi system (Bio-Rad) at 25 °C with 2× buffer solution [16] in the top chamber (0.38 M glycine, 0.5M Tris, 0.2% SDS, pH 8.3) and a 1× buffer solution in the bottom chamber running at 35 mA/gel for approximately 1000 Vh. Proteins were visualized by staining gels with colloidal Coomassie blue [17]. Briefly, gels were fixed overnight in 50% ethanol and 3% phosphoric acid, followed by 3 washes with dH_2O. Gels were pre-stained for 1 h in 34% methanol, 17% ammonium sulfate and 3% phosphoric acid and finally stained in the same solution containing Coomassie blue G-250 (0.066%) for 3 days. The gels were rinsed and stored in a 10% ammonium sulfate solution and scanned using laser densitometry (PDSI, GE Healthcare). Two-to-four gels were run for each condition (pH range and fraction) and spots were picked from a representative gel.

2.4. In-gel digestion of proteins

Protein spots were excised from the stained gel and stored in dH_20 at −80 °C. Thawed gel plugs were first de-stained with a standard gel de-staining solution (dH_20:methanol:acetic acid [4.5:4.5:1]), rinsed in dH_20 (5 min) and finally de-stained in 50% ACN containing 25 mM ammonium bicarbonate (3 min × 15 min). The gel plugs were dehydrated with 100% ACN (5 min), dried under vacuum, and then re-swollen with 10 or 20 μl of 10 μg/ml trypsin (modified porcine trypsin, sequencing grade, Promega, Madison, WI) in 25 mM ammonium bicarbonate, pH 8.0. An additional 30 μl 25 mM ammonium bicarbonate buffer was added to cover the gel plugs. Digestion was performed overnight at 37 °C. The resulting tryptic fragments were extracted with 5% TFA in 50% ACN with sonication for 60 min, twice. The extracts were dried to completeness by vacuum centrifugation and then dissolved in 50% ACN and 0.1% TFA.

2.5. Protein identification

2.5.1. Mass spectrometry

A matrix-assisted laser desorption/ionization-time of flight mass spectrometer (Voyager DE-STR MALDI–TOF–MS, Applied Biosystems, Framingham, MA) operated in positive ion reflector mode was used to analyze tryptic peptides by peptide mass fingerprinting (PMF). Samples (0.5 μl) were co-crystallized with freshly prepared a-cyanohydroxycinnamic acid (re-crystalized from Sigma #C-2020) matrix in 50% ACN and 0.1% TFA (0.5 μl) directly onto a target plate. Spotted samples were further desalted by addition of 0.1% TFA (3 μl) which was immediately removed by vacuum. Spectra were acquired with 75 shots of a 337 nm Nitrogen Laser operating at 20 Hz and were calibrated using the porcine trypsin autolysis peaks at *m/z* 842.51 and 2,211.10 as internal standards. Samples which were not initially identified by PMF were vacuum-dried, re-suspended in 20 μl 5% ACN, 0.1% formic acid and subjected to MS/MS analysis (Thermo Finnigan LCQ DecaXP plus ion trap mass spectrometer, San Jose, CA). Peptides were separated by reverse phase chromatography using a 30 min 5–60% ACN gradient in 0.1% TFA. The instrument was operated with a duty cycle that acquired MS/MS spectra on the three most abundant ions following a survey scan from 400 to 1600 kDa. Dynamic exclusion was employed to prevent the continuous analysis of the same ions. Once two MS/MS spectra had been acquired from any given ion, the parent mass was placed on an exclusion list for the duration of 1.5 min. The raw data were processed by Sequest to generate DTA files for database searching. The merge.pl script from Matrix Science was used to convert Sequest DTA files into a single generic file for searching in Mascot.

2.5.2. Data analysis

Protein identification by PMF was performed by searching the National Center for Biotechnology Information non-redundant (NCBInr) and SwissProt primary databases using the Mascot search engine (http://www.matrixscience.com), which uses a probability based scoring system [18]. The following parameters were used for database searches: monoisotopic mass, 25 ppm mass accuracy, trypsin as digesting enzyme with 1 missed cleavage allowed and carbamidomethylation of cysteine, oxidation of methionine, N-terminal pyroglutamic acid from glutamic acid or glutamine as allowable variable modifications; taxonomy was limited to Mammalia. To qualify the MALDI–TOF–MS data as a positive identification, a protein's score (MOWSE) was equal to or exceeded the minimum significant score (70 and 60 for NCBInr and SwissProt, respectively). For LC MS/MS analyses, if the primary databases did not yield a positive identification, "EST_others" was queried and significant sequence matches to swine ESTs were further Blasted to the primary databases. Positive identification of proteins by MS/MS analysis required a minimum of two unique peptides, both with significant ion scores and with an apparent molecular weight within approximately 10 kDa of the expected mass of each protein.

3. Results and discussion

Preliminary 2D-PAGE separation of porcine liver proteins using full-length (pH 3–10) IPG strips revealed that the vast majority of proteins were observed between pH 5 and 9 (data not shown) and using multiple narrow range IPG strips (pH 3–6, 5–8 and 7–10), the present study has confirmed those observations. Similar findings in the p*I* range of most observable proteins by 2D-PAGE have been reported for rat liver [19]. Because separation of proteins in the alkaline range is notoriously poor due to DTT migration we have employed paper-bridge loading with additional DTT at the cathodic electrode to improve separation of proteins in the pH 7–10 range

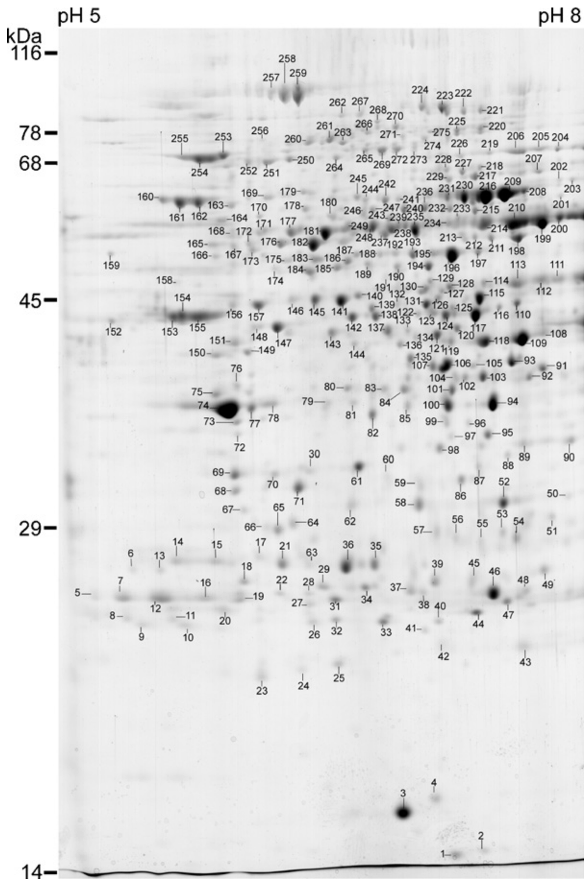

Fig. 1. Colloidal Coomassie stained 2D-PAGE of porcine hepatocyte cytosol proteins. Proteins were separated in the first dimension on a pH 5–8, 11 cm IPG strip. The numbered spots were prepared for MS analysis and the combined data are presented in Table 1 as gel # 1.

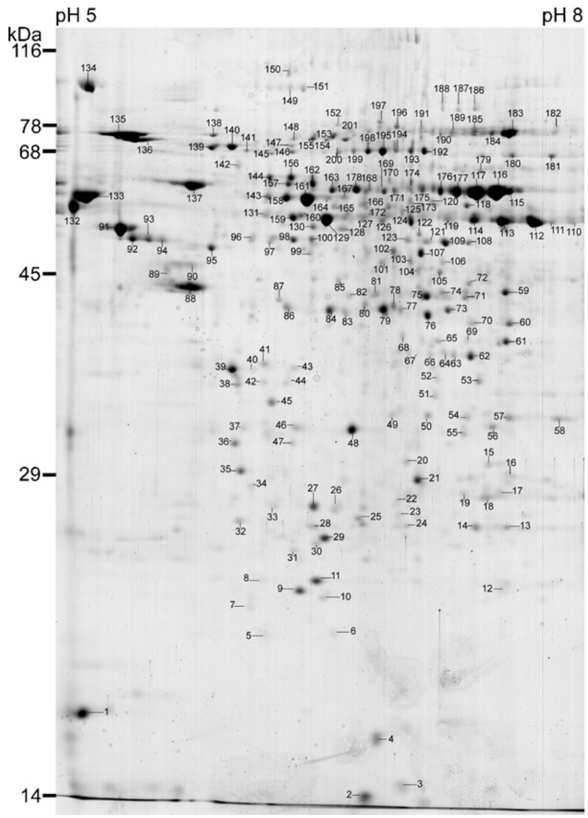

Fig. 2. Colloidal Coomassie stained 2D-PAGE of porcine hepatocyte membrane proteins. Proteins were separated in the first dimension on a pH 5–8, 11 cm IPG strip. The numbered spots were prepared for MS analysis and the combined data are presented in Table 1 as gel # 2.

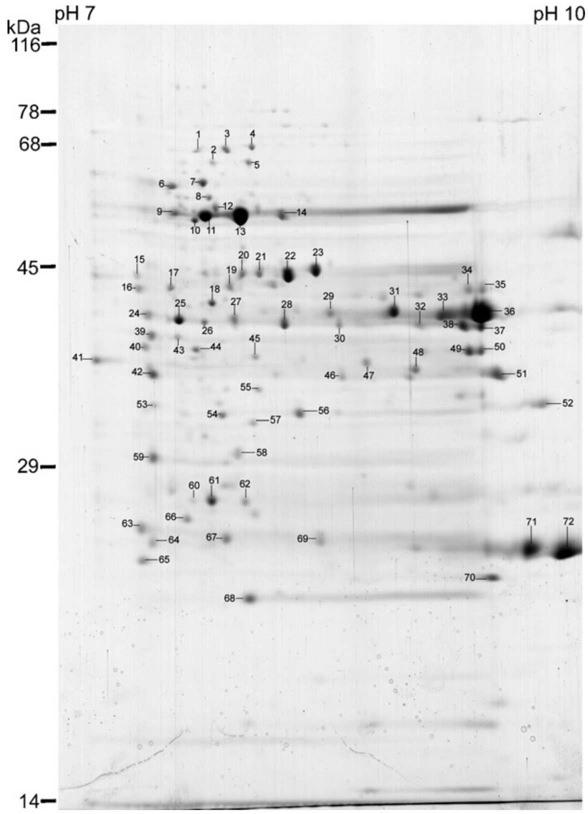

Fig. 3. Colloidal Coomassie stained 2D-PAGE of porcine hepatocyte cytosol proteins. Proteins were separated in the first dimension on a pH 7–10, 11 cm IPG strip. The numbered spots were prepared for MS analysis and the combined data are presented in Table 1 as gel # 3.

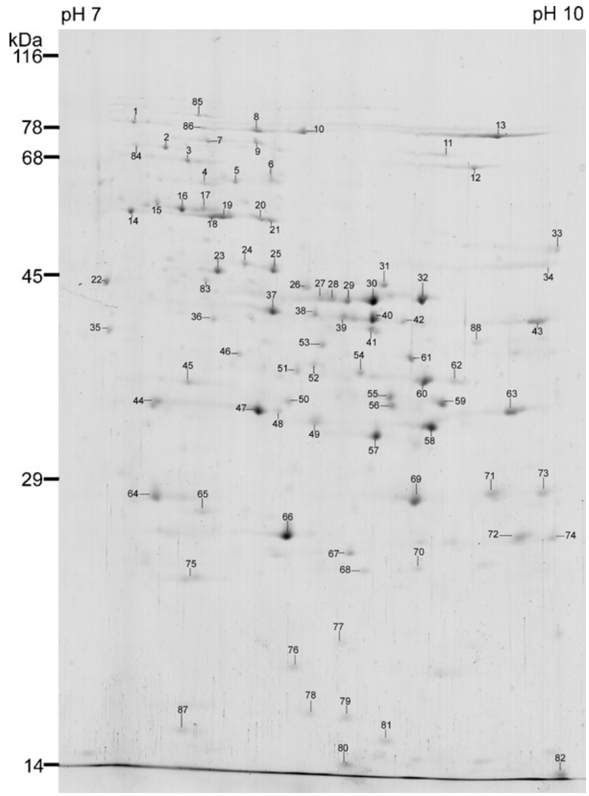

Fig. 4. Colloidal Coomassie stained 2D-PAGE of porcine hepatocyte membrane proteins. Proteins were separated in the first dimension on a pH 7–10, 11 cm IPG strip. The numbered spots were prepared for MS analysis and the combined data are presented in Table 1 as gel # 4.

[14]. In our hands, standard in-gel re-hydration protein loading yielded poor separation for many proteins and this modification was absolutely necessary to yield consistent separation in the alkaline pH range.

Representative annotated gels are shown in Figs. 1–6 (viz., gel 1, pH 5–8 cytosol; gel 2, pH 5–8 membranes; gel 3, pH 7–10 cytosol; gel 4, pH 7–10 membranes; gel 5, pH 3–6 cytosol and gel 6, pH 3–6 membranes). A total of 728 spots were picked and analyzed by mass spectrometry and 648 protein assignments were made. The detailed identification of each individual protein spot from these gels is included as supplemental data (Supplement 1), while summation of all unique proteins and their location on each gel map are provided in Table 1. As listed, 282 unique proteins were identified and represent the products of 280 unique genes. The noted exceptions were 17-beta estradiol dehydrogenase where both the 80 and 32 kDa active isoforms [20] were detected in gel 4, and multiple N-terminal and a C-terminal domains of carbamoyl phosphate synthetase were observed in both the membrane and cytosolic gels. The full-length 164 kDa protein was not observed and in all cases peptides encompassing either the C-terminal or N-terminal domains, but not both, were noted. These lower 60–80 kDa molecular weight isoforms of carbamoyl phosphate synthetase have been recognized in baboon liver and are apparently up-regulated during inflammation [21]. Additional multiple isoforms (or spots) of various proteins appearing in either fraction and across gels are a combination of post-translational modifications, other potential proteolytic products (precursor vs. mature), and may also be the result of the crude membrane-cytosol preparation with frozen cells.

By utilizing MALDI–TOF–MS (PMF) as an initial screening tool, 349 or 53.9% of the 648 proteins were positively identified. Of those, only 167 (47.9%) were assignments based on *Sus scrofa* identities which indicates that as more sequences and annotations from pig are available in public domain databases, higher percentages of identifications by PMF could be expected. Only those spots which could not be positively identified by PMF were prepared for ESI-MS/MS analysis. Of the potential 379 spots which were not identified

Table 1
Summary of 282 unique protein assignments of porcine hepatocyte cytosol and membrane fractions in six different gels: gel 1, cytosol pH 5–8; gel 2, membranes pH 5–8; gel 3, cytosol pH 7–10, gel 4, membranes pH 7–10; gel 5, cytosol pH 3–6 and gel 6, membranes pH 3–6

Gel and spot number	Protein identification	Representative accession no.
5-25	14-3-3 Protein beta/alpha (Protein kinase C inhibitor protein 1) (KCIP-1)	1433B_BOVIN
5-4	14-3-3 Protein gamma (Protein kinase C inhibitor protein 1)	108451
3-48	15-Oxoprostaglandin 13-reductase	2947100
4-8,10	17-Beta-estradiol dehydrogenase	47523670
4-50,56	17-Beta-estradiol dehydrogenase (N-terminal active 32 kDa dehydrogenase)	1090092
4-86	17-Beta-hydroxysteroid dehydrogenase 4	DHB4_MOUSE
3-52; 4-63	2,4-Dienoyl-CoA reductase	37934198
4-70	2-Cys Peroxiredoxin, Hbp23	6435547
2-101	2-Oxoisovalerate dehydrogenase subunit alpha, mitochondrial precursor	ODBA_BOVIN
4-65	3,2-*trans*-Enoyl-CoA isomerase, mitochondrial (D3D2_HUMAN) 1099293 MARC 4PIG Sus scrofa cDNA 3′	59777590
4-42	3,2-*trans*-Enoyl-CoA isomerase, PECI-peroxisomal (PECI_HUMAN) CJ000466 swine cDNA clone LVR01E040088, 5′	54483061
3-69; 4-66	3-Hydroxyacyl-CoA dehydrogenase type-2	HCD2_BOVIN
1-71	3-Hydroxyanthranilate 3,4-dioxygenase	3HAO_RAT
1-56	3-Hydroxybutyrate dehydrogenase type 2	BDH2_BOVIN
1-68; 2-36; 6-10	3-Hydroxyisobutyrate dehydrogenase	3HIDH_RAT
4-27, 28,29,30	3-Ketoacyl-CoA thiolase	THIK_HUMAN
4-30	3-Ketoacyl-CoA thiolase A	THIKA_RAT
4-34	3-Ketoacyl-CoA thiolase, long chain	47522760
3-34, 35; 4-32	3-Ketoacyl-CoA thiolase, mitochondrial	THIM_RAT
1-85, 94,100; 3-42	3-Oxo-5-beta-steroid 4-dehydrogenase	AK1D1_HUMAN
1-210	3-Phosphoglycerate dehydrogenase	13928850
5-8; 6-7	40S ribosomal protein SA (p40) (34/67 kDa laminin receptor)	RSSA_CRIGR
1-141,145,146	4-Hydroxyphenylpyruvic acid dioxygenase	47523532
1-172,176, 182; 2-130	4-Trimethylaminobutyraldehyde dehydrogenase (Aldehyde DH 9A1)	AL9A1_BOVIN
2-41,43	60S acidic ribosomal protein P0 (L10E)	RLA0_BOVIN
4-29	Acetyl-CoA acetyltransferase (Acetoacetyl-CoA thiolase)	THIL_MOUSE
1-223,224	Aconitase (Iron regulatory protein-1)	IREB1_RABIT
4-85	Aconitate hydratase, mitochondrial precursor (Aconitase)	ACON_PIG
2-88	Actin (gamma)	809561
1-76, 153,154,155; 5-40,41, 53,54, 60, 62; 6-12, 13	Actin, cytoplasmic 1 (Beta-actin)	ACTB_BOVIN
1-174	Actin-like protein 3 (Actin-related protein 3)	ARP3_MOUSE
3-5	Acyl CoA synthetase, medium chain (Predicted) [gi:76653089, Bos taurus] 1518577 MARC 3PIG cDNA 3′	87214063
4-7	Acyl coenzyme A synthetase, long-chain 1	50604627
1-121,137; 2-76, 2-79	Acyl-CoA dehydrogenase (Butyryl-CoA DH-short chain)	ACADS_PIG
2-71	Acyl-Coa dehydrogenase, chain A, medium-chain	40889734
1-123; 2-81	Acyl-Coa dehydrogenase, medium chain, chain B	640353
1-117; 2-75	Acyl-CoA dehydrogenase, medium-chain specific	ACADM_PIG
1-120,137,143; 2-79,83,84,86	Acyl-CoA dehydrogenase, short/branched chain specific	ACDSB_RAT
1-133	Acyl-Coenzyme A dehydrogenase family, member 8	7656849

Table 1 (*Continued*)

Gel and spot number	Protein identification	Representative accession no.
4-9	Acyl-coenzyme A oxidase 1 l(Palmitoyl-CoA oxidase)	ACOX1_HUMAN
1-139	Adenosine kinase isoform b isoform 1 (Predicted)	57085123
1-92	Aflatoxin B1 aldehyde reductase member 4	ARK74_HUMAN
1-61; 2-46,48	Agmatinase, mitochondrial precursor (Agmatine ureohydrolase)	SPEB_HUMAN
1-195,197; 2-108	Alanine–glyoxylate aminotransferase 2, mitochondrial precursor	AGT2_RAT
1-178,264; 2-195,198,199,200	Albumin	833798
3-31,32,33,36; 4-38,40,41	Alcohol dehydrogenase 1	ADH1_MOUSE
3-28; 4-36	Alcohol dehydrogenase class II isozyme 1	3912976
1-257,258,259; 2-149,151	Aldehyde dehydrogenase 1 family, member L1 (10-FTHFDH)	FTHFD_MOUSE
1-259	Aldehyde dehydrogenase 1 family, member L2	77681579
1-171,177,181, 238, 248; 2-127,129,131,159,160,165	Aldehyde dehydrogenase 2, mitochondrial	113205888
3-12	Aldehyde dehydrogenase family 6, subfamily A1 (Aldh6a1)	21410418
1-235,237,238; 2-122,126	Aldehyde dehydrogenase family 7 member A1 (Antiquitin-1)	AL7A1_HUMAN
1-91; 3-44	Aldehyde Reductase (aldo-keto reductase family 1 member A1)	AK1A1_PIG
3-46	Aldo-keto reductase family 1 member C1, putative	113205730
1-188,193,196; 2-109	Alpha enolase	ENOA_BOVIN
1-147,148; 2-87	Aminoacylase I	1845
5-18	Annexin A5 (Annexin V) (Lipocortin V) (Endonexin II) (Calphobindin I) (CBP-I)	ANXA5_BOVIN
1-250	Annexin VI	1842109
2-45	Apolipoprotein E	47523674
1-106; 2-65	Arginase I	15281140
1-188	Argininosuccinate lyase (Arginosuccinase) (ASAL)	114160
1-111,112; 3-21,23; 4-25	Argininosuccinate synthase	ASSY_RAT
4-43	Aspartate aminotransferase	47522630
3-18	Aspartate aminotransferase, cytosolic	47522636
1-159; 2-91; 5-24; 6-17,18	ATP synthase H + transporting F1 complex beta subunit	89574051
2-11	ATP synthase, mitochondrial F0 complex, subunit d	27807305
1-111,112,113; 3-20,22; 4-23	Betaine–homocysteine *S*-methyltransferase	BHMT_MOUSE
1-138,142	Beta-ureidopropionase (Beta-alanine synthase)	BUP1_PONPY
1-185	Bile acid CoA:amino acid *N*-acyltransferase (BAAT_HUMAN) MI-P-CP1-nzc-l-24-0-UI.s1 MI-P-CP1 3′cDNA.	21551454
1-44; 3-65	Biliverdin reductase B (flavin reductase (NADPH)	BLVRB_MOUSE
4-11	Calcium-binding mitochondrial carrier protein Aralar2	CMC2_HUMAN
2-132; 5-22	Calreticulin precursor (CRP55) (partial sequence)	CALR_PIG
1-261,266,270,274; 2-153,196,197	Carbamoyl-phosphate synthetase (∼80 kDa N-terminal domain)	CPSM_MOUSE
2-147,148,155	Carbamoyl-phosphate synthetase 1 (∼70 kDa N-terminal domain)	8393186
2-142	Carbamoyl-phosphate synthetase 1 (∼65 kDa N-terminal domain)	8393186
1-220,225,228,245,272,273, 275; 2-185	Carbamoyl-phosphate synthetase I (C-terminal domain)	26324620
3-58	Carbonyl reductase [NADPH] 20-beta-hydroxysteroid DH)	DHCA_PIG
4-71	Carbonyl reductase/NADP-retinol dehydrogenase	47522860

Table 1 (*Continued*)

Gel and spot number	Protein identification	Representative accession no.
2-144,156,157,162,163, 168,178	Carboxylesterase precursor (Proline-beta-naphthylamidase)	EST1_PIG
4-12	Carnitine *O*-palmitoyltransferase 2	CPT2_MACFA
1-208,209,216,230,231; 2-115, 116,174,175,176,177; 3-6	Catalase	12082093
1-7,12,20; 5-28,29,30	Catechol *O*-methyltransferase	COMT_PIG
1-57	Cathepsin D	56417363
1-163	Chaperonin containing TCP1, subunit 8 (theta)	73586947
4-5	Choline dehydrogenase	27501456
5-19	Coatomer subunit epsilon (Epsilon-coat protein)	COPE_BOVIN
1-116,117	Cystathionase	104295139
2-1; 6-2,3	Cytochrome b5	CYB5_PIG
2-3	Cytochrome C oxidase subunit 5B, mitochondrial precursor	COX5B_PIG
1-152; 5-41,42,43, 44,52	Cytokeratin 18	K1C18_MOUSE
1-158,166; 2-89,160; 5-44	Cytokeratin 8	K2C8_BOVIN
1-75	cytosolic inorganic pyrophosphatase	4583153
2-61,69; 3-43; 4-35	D-Amino acid oxidase	66086
4-57	D-Beta-hydroxybutyrate dehydrogenase	203921
4-51	Dehydrogenase/reductase SDR member 1 (DHRS1_MOUSE) BW981110 swine cDNA clone ITT010057H07, 5′	74257950
1-101	Delta-aminolevulinic acid dehydratase isoform b	86438477
2-118	Dihydrolipoyl dehydrogenase, mitochondrial precursor	DLDH_PIG
2-100	Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, mitochondrial precursor	ODO2_PIG
1-202,203; 3-7	Dihydroxyacetone kinase	DAK_BOVIN
1-135	dimeric dihydrodiol dehydrogenase	47523420
1-221,222; 2-186, 188	Dimethylglycine dehydrogenase mitochondrial precursor	M2GD_HUMAN
1-26,33	DJ-1 protein (Type 1 glutamine amidotransferase)	67038668
2-78	DnaJ (Hsp40) homolog, subfamily B, member 11	77735491
3-56; 4-47,48	Electron transfer flavoprotein alpha subunit precursor	35384836
4-69	Electron transfer flavoprotein subunit beta	ETFB_PIG
2-179	Electron transfer flavoprotein-ubiquinone oxidoreductase, mitochondrial precursor	ETFD_PIG
5-20	Elongation factor 1 delta (translational)	57164211
5-5	Elongation factor 1-beta (EF-1-beta)	EF1B_BOVIN
1-128; 2-105	Elongation factor Tu, mitochondrial precursor	EFTU_BOVIN
2-21	Endoplasmic reticulum protein 29 isoform 1 precursor	ERP29_HUMAN
1-87	Enoyl coenzyme A hydratase 1, peroxisomal	90024980
2-18; 3-60,61; 4-64	Enoyl-CoA hydratase, mitochondrial precursor	ECHM_BOVIN
4-13	Enoyl-Coenzyme A hydratase/3-hydroxyacyl Coenzyme A	51493747
1-241,242,244; 2-169	Epoxide hydrolase, soluble	HYES_PIG
1-80	Ester hydrolase C11orf54 homolog (BW960903 full-length enriched swine cDNA)	71961233
1-95,97; 3-53	Esterase D (*S*-formylglutathione hydrolase)	ESTD_PIG
5-1	Eukaryotic translation initiation factor 5A-1 (eIF-5A-1) (eIF-5A1)	IF5A1_BOVIN
5-15	Eukaryotic translation initiation factor 6 (eIF-6)	IF6_BOVIN
4-18,19,20,21	F1-Atpase, chain A, mitchondrial	1943080
2-41	F-actin capping protein alpha-1 subunit (Predicted)	61821551
2-2	Fatty acid binding protein-liver	FABPL_PIG

Table 1 (*Continued*)

Gel and spot number	Protein identification	Representative accession no.
1-23	Ferritin heavy chain (Ferritin H subunit)	47522776
1-25	Ferritin L subunit	10304378
1-236,240,247	Formiminotransferase-cyclodeaminase	47523624
1-93,105,107;3-39;5-65	Fructose-1,6-bisphosphatase 1 (EC 3.1.3.11)	F16P1_PIG
3-32,37,38; 4-41	Fructose-bisphosphate aldolase B	ALDOB_HUMAN
1-117,122,124; 3-16	Fumarylacetoacetase (Beta-diketonase)	FAAA_HUMAN
1-98; 2-50	Fumarylacetoacetate hydrolase domain containing 2A	7705608
1-149	Galactokinase 1	110665600
1-134	GDP-mannose pyrophosphorylase B	29244556
2-141	Glucose regulated protein 75 (Stress-70 protein), mitochondrial precursor	GRP75_HUMAN
2-135; 5-46; 6-21	Glucose-regulated protein precursor (GRP 78) (Heat shock 70 kDa protein 5)	GRP78_MESAU
2-150	Glucosidase II	47522680
1-199,210,214,233, 234,239; 2-110,111, 112,113,114,119,125; 3-9,10; 4-14	Glutamate dehydrogenase 1, mitochondrial precursor	DHE3_HUMAN
1-130,131	Glutamine synthetase	73586743
4-83	Glutaryl-CoA dehydrogenase, mitochondrial precursor	GCDH_PIG
1-27	Glutathione peroxidase, cytosolic	47523488
1-19, 37,46; 2-14; 3-63,71,72; 4-72	Glutathione *S*-transferase (alpha M14)	GSTA1_PIG
1-52,58,62; 2-15,20; 3-59	Glutathione *S*-transferase (omega-1)	GSTO1_PIG
4-67	Glutathione *S*-transferase kappa 1 (Predicted)	73978758
3-64,67	Glutathione *S*-transferase mu 2	116047847
3-64	Glutathione *S*-transferase mu 5	23065563
1-17,19,38,47,48	Glutathione *S*-transferase Y1 (Chain 3) (GST class-mu)	399829
1-173	Glutathione synthetase	4504169
3-49,50; 4-61	Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	G3P_PIG
2-128	Glycerate kinase (hypothetical 54 kDa protein, LOC507949)	114050933
1-83,84	Glycerol-3-phosphate dehydrogenase, cytosolic	2149959
2-64	Glycerophosphate dehydrogenase (KIAA0089)	577307
1-129,189,190,194; 2-102,107	Glycine amidinotransferase (L-arginine:glycine amidinotransferase)	GATM_PIG
4-45	Glycine-*N*-acyltransferase [gi:29135315], Bos taurus, BP139133 swine cDNA library clone LVRM10079D06, 5′	40386874
2-31	GTP-binding protein SAR1b	SAR1B_BOVIN
1-14,15; 5-27	Guanidinoacetate *N*-methyltransferase	GAMT_BOVIN
1-184; 2-99	Guanosine diphosphate dissociation inhibitor 2	48675953
1-18; 2-24,28,32	Heat shock 27 kDa protein 1	50979116
1-251,252; 2-145	Heat shock 70 kDa protein 1A (HSP70.1)	HS70A_BOVIN
1-254,255; 2-139; 5-49,51	Heat shock cognate 71 kDa protein (Heat shock 70 kDa protein 8)	HSP7C_HUMAN
1-160,161,162;2-137; 5-26,56, 57; 6-23	Heat shock protein (Hsp60)	CH60_HUMAN
1-256	Heat shock protein (HSP90)	47522774
1-253; 2-140; 6-24	Heat shock protein 9A (GRP75)	6754256
2-134	Heat shock protein (HSP 90 kDa beta member 1, GRP94)	ENPL_PIG
1-20	Heme-binding protein	58332866
4-54,62	Heterogeneous nuclear ribonucleoprotein A2/B1 isoform 2	32880197

Table 1 (*Continued*)

Gel and spot number	Protein identification	Representative accession no.
4-24	Hydroxy steroid dehydrogenase like 2 [gi:13195670] BP151282 swine cDNA clone OVRM10072F02, 5′	40400755
4-37	Hydroxyacid oxidase 1 (Glycolate oxidase)	HAOX1_MOUSE
4-53	Hydroxyacid oxidase 2 (peroxisomal, long chain)	HAOX2_HUMAN
1-51	Hydroxyacyl glutathione hydrolase	GLO2_MOUSE
4-59	Hydroxyacyl-coenzyme A dehydrogenase, mitochondrial precursor	HCDH_PIG
1-164;5-68	Hydroxymethylglutaryl-CoA synthase, cytoplasmic	HMCS1_HUMAN
1-39	Hypoxanthine-guanine phosphoribosyltransferase	2499938
5-34	Indolethylamine *N*-methyltransferase (INMT_MOUSE) BP443228 swine cDNA clone LVR010100C06 5′	40433295
1-115,126,127;2-72;4-31	Isocitrate dehydrogenase, NADP dependent	6647554
1-35; 2-19	Isopentenyldiphosphate isomerase	48257093
1-110;2-59; 3-17; 4-22	Isovaleryl-CoA dehydrogenase, mitochondrial precursor	IVD_HUMAN
1-69	Ketohexokinase (KHK_MOUSE) MPL384_4_A17 MPL swine cDNA clone pSPORT1, 5′)	26018027
2-56	Lactamase, beta 2	7705793
1-8, 11; 5-31	Lactoylglutathione lyase (Glyoxalase I)	LGUL_HUMAN
1-50	L-Aspartate dehydrogenase (putative)	ASPD_MOUSE
1-239,248	Leucine aminopeptidase) (LAP, cytosolic)	AMPL_BOVIN
1-136	Leukocyte elastase inhibitor (LEI) Serpin B1	417185
1-77	L-Lactate dehydrogenase B chain (LDH-B)	1170738
1-82	Malate dehydrogenase	164543
3-51; 4-60	Malate dehydrogenase, mitochondrial	65932
1-86	MAWD Binding Protein	19743770
4-15,17	Methylmalonate-semialdehyde dehydrogenase	MMSA_RAT
4-81	Mitochondrial fission 1 protein (Fis1 homolog, Tetratricopeptide repeat protein 11)	FIS1_RAT
2-192,193	Mitochondrial respiratory complex 1i (succinate dehydrogenase) chain A	73535956
4-6	Monoamine oxidase B	49274643
2-138	NADH dehydrogenase (ubiquinone) Fe-S protein 1, 75 kDa precursor	33519475
2-30	NADH dehydrogenase [ubiquinone] flavoprotein 2, mitochondrial precursor	NDUV2_MOUSE
2-34	NADH dehydrogenase Fe-S protein 3	20071222
1-42,43;3-68	Neuropolypeptide h3 (Phosphatidylethanolamine-Binding Protein)	913159
1-73; 5-37	Nicotinate-nucleotide pyrophosphorylase (NADC_BOVIN) 1113707 MARC 4PIG Sus scrofa cDNA 5′	59808271
1-21,36	Non-selenium glutathione phospholipid hydroperoxide peroxidase (PHGPx)	6689393
1-102,103, 104; 2-64,66,67; 2-62,63; 3-40	Ornithine carbamoyltransferase, mitochondrial precursor	OTC_PIG
4-79	Peptidyl-prolyl *cis-trans* isomerase A (Rotamase A, Cyclophilin A)	PPIA_AOTTR
3-70	Peroxiredoxin 1	6754976
1-9,10; 5-32	Peroxiredoxin-2 (Thioredoxin peroxidase 1)	1717797
2-25,29	Peroxiredoxin-3 (Thioredoxin-dependent peroxide reductase)	PRDX3_BOVIN
2-27	Peroxiredoxin-4 (thioredoxin peroxidase)	5453549
1-4	Peroxiredoxin-5	10305336
2-26,33	Peroxiredoxin-6 (non-selenium glutathione peroxidase)	PRDX6_PIG
1-70	Phenol sulfotransferase	37780220

Table 1 (*Continued*)

Gel and spot number	Protein identification	Representative accession no.
1-167	Phenylalanine-4-hydroxylase	PH4H_HUMAN
1-204,206, 219; 2-180,181; 3-3; 4-2	Phosphoenolpyruvate carboxykinase [GTP]	PPCKM_MOUSE
1-207, 217, 229	Phosphoglucomutase-1	PGM1_HUMAN
1-54	Phosphoglycerate mutase 1	44890768
5-55	Phosphomannomutase 2	PMM2_BOVIN
3-27	phosphoserine aminotransferase isoform 1	17402893
4-80	Profilin-1	PROF1_BOVIN
2-35; 6-9	Prohibitin	PHB_BOVIN
1-180	Proline dipeptidase (Predicted)	76640954
2-133; 5-10; 6-20	Prolyl 4-hydroxylase subunit beta (thyroid hormone binding protein)	PDIA1_BOVIN
2-103,106	Propanoyl-CoA C-acyltransferase (NLTP_RABIT) BP451909 swine cDNA library, clone LVRM10013E09 5′	40441976
1-263; 2-154,201	Propionyl-CoA carboxylase alpha chain, mitochondrial precursor	PCCA_MOUSE
1-243; 2-166	Propionyl-CoA carboxylase beta chain precursor	47522682
1-59	Prosomal protein P30-33K	190447
3-45	Prostaglandin F synthase (aldo-keto reductase family 1 member C4)	61741954
5-67	Proteasome 26S non-ATPase regulatory subunit S5 (Predicted)	73971550
5-59	Proteasome 26S subunit ATPase 3,(26S protease regulatory subunit 6A)	PRS6A_HUMAN
5-50	Proteasome 26S subunit ATPase 4 (26S protease regulatory subunit 6B)	PRS6B_BOVIN
1-64	Proteasome activator 28 alpha subunit	34978646
1-67	Proteasome activator 28 beta subunit	34978648
1-28	Proteasome beta 3 subunit	6755202
5-14	Proteasome subunit alpha type 5	PSA5_HUMAN
1-41	Proteasome subunit beta type 2	PSB2_BOVIN
5-2	Proteasome subunit beta type 6 precursor (delta chain)	PSB6_HUMAN
1-22	proteasome subunit, beta type 4	77735487
2-92; 6-16	Protein disulfide isomerase related protein 5	1710248
1-178; 2-143, 158,161,164	Protein disulfide-isomerase A3 precursor (ERp60, 58 kDa microsomal protein)	PDIA3_HUMAN
2-136	Protein disulfide-isomerase A4 precursor	PDIA4_HUMAN
3-62	Quinoid dihydropteridine reductase	47523746
1-74,75,78,79,80; 2-39;3-41; 6-11	Regucalcin	116175265
1-200,210, 233,238,239; 3-9,11,13; 4-16	Retinal dehydrogenase 1 (ADH family 1 member A1)	AL1A1_HORSE
2-9	Retinol-binding protein precursor (PRBP)	RETBP_PIG
1-6,13	Rho Family Gtp-Binding Protein Cdc42, chain b	7245833
5-33	Rho GDP-dissociation inhibitor 1 (Rho GDI 1, alpha)	GDIR_BOVIN
1-89,90;2-57,58;3-54; 4-44	Rhodanese	THTR_BOVIN
1-99	Ribose-phosphate pyrophosphokinase II (PRS-II) isoform 3	PRPS2_HUMAN
1-140	*S*-Adenosylhomocysteine hydrolase	58801555
1-183,186	*S*-Adenosylmethionine synthetase isoform type-1	METK1_RAT
3-19	SEC14-like protein 2 (Alpha-tocopherol-associated protein)	S14L2_BOVIN
1-156,157	SEC14-like protein 3 (45 kDa secretory protein)	S14L3_RAT
1-233,235,237,238,239, 248,	Selenium binding protein 1	SBP1_HUMAN
1-235,238	Selenium-binding protein 2 (56 kDa acetaminophen-binding protein)	SBP2_MOUSE

Table 1 (*Continued*)

Gel and spot number	Protein identification	Representative accession no.
1-198,211,212	Serine hydroxymethyltransferase, cytosolic	GLYC_RABIT
5-47	Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha isoform	2AAA_PIG
1-65	Short-chain dehydrogenase/reductase	27807265
1-108,109,118,119; 2-60; 3-24,25,32	Sorbitol dehydrogenase	DHSO_BOVIN
4-4,82	Sterol carrier protein-2	200942
1-31	Substrate protein of ATP-dependent proteinase SP-22 (peroxiredoxin family)	627764
4-69	Succinate dehydrogenase [ubiquinone] mitochondrial precursor	DHSB_BOVIN
5-61; 6-12	Succinyl-CoA ligase [GDP-forming] beta-chain, mitochondrial precursor	SUCB2_PIG
1-3; 2-4	Superoxide dismutase [Cu-Zn]	SODC_PIG
1-179	T-Complex protein 1 subunit alpha B (TCP-1-alpha)	TCPA2_MOUSE
1-235,238,239; 2-125	T-Complex protein 1 subunit beta (TCP-1-beta	TCPB_BOVIN
1-227	T-Complex protein 1 subunit gamma (TCP-1-gamma)-chaparonin	TCPG_BOVIN
1-229	T-Complex protein 1 subunit zeta (TCP-1-zeta)	TCPZ_BOVIN
2-90	Thioredoxin domain containing 4 (endoplasmic reticulum)	78042524
2-29	Thioredoxin-dependent peroxide reductase (Predicted) confirmed by MS/MS	73998671
4-55	*trans*-2-Enoyl-CoA reductase, peroxisomal (PECR_RAT) BP141613 swine cDNA clone OVR010044A10, 5′	40391084
2-183,184,185; 4-1	Transferrin	TRFE_PIG
5-48,56	Transitional endoplasmic reticulum ATPase (TER, 15S Mg(2+)-ATPase p97 subunit)	TERA_PIG
1-205; 3-3,4	Transketolase	TKT_BOVIN
5-3	Translationally controlled tumor protein (TCTP)	TCTP_PIG
1-45,49; 3-66	Triosephosphate isomerase 1	91214448
5-7	Tropomyosin 3 isoform 2 isoform 3 (Predicted)	73961067
5-12	Tubulin alpha-3 chain	TBA3_CRIGR
5-58	Tubulin alpha-8 chain (Alpha-tubulin 8)	TBA8_HUMAN
5-11,38,39; 6-19	Tubulin beta-5 chain (Beta-tubulin isotype I) (Class I beta tubulin)	TBB5_CRIGR
5-6	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, epsilon	31981925
4-26	Ubiquinol-cytochrome-C reductase complex core protein 2	UQCR2_BOVIN
2-95; 6-15	Ubiquinol-cytochrome-C reductase core protein I, mitochondrial precursor	UQCR1_HUMAN
1-200; 3-13,14	UDP-glucose pyrophosphorylase -2	UGPA2_PIG
1-201,232; 3-8	UDP-glucose 6-dehydrogenase	UGDH_HUMAN
1-210	UDP-glucose pyrophosphorylase-1(UGPA1)	731050
1-33	UMP-CMP kinase (Cytidylate kinase, Deoxycytidylate kinase)	2497487
1-269	Urocanase domain containing 1 (Predicted)	76622316
2-49	Voltage-dependent anion channel VDAC2 (outer mitochondrial membrane)	346412
4-49,58	Voltage-dependent anion-selective channel protein 1 (VDAC-1)	VDAC1_PIG
1-226	WDR1 protein	3420181
4-52	Zeta-crystallin (Quinone oxidoreductase)	113205780
1-150; 5-63,64	Zinc-binding alcohol dehydrogenase domain-containing protein 1	ZADH1_HUMAN

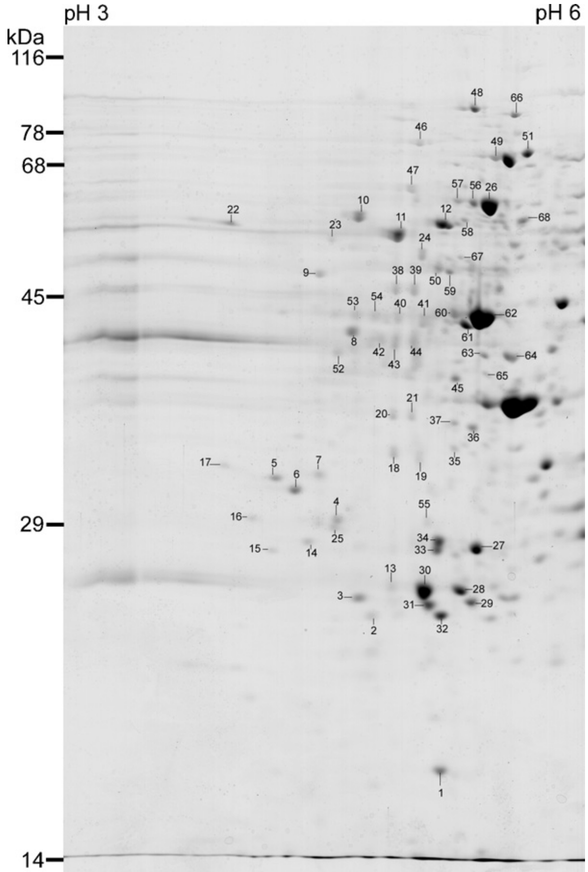

Fig. 5. Colloidal Coomassie stained 2D-PAGE of porcine hepatocyte cytosol proteins. Proteins were separated in the first dimension on a pH 3–6, 11 cm IPG strip. The numbered spots were prepared for MS analysis and the combined data are presented in Table 1 as gel # 5.

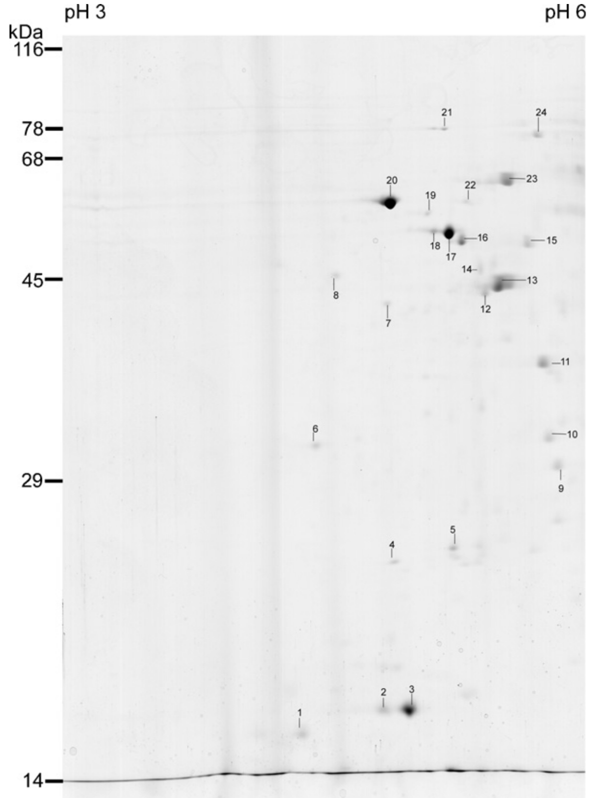

Fig. 6. Colloidal Coomassie stained 2D-PAGE of porcine hepatocyte membrane proteins. Proteins were separated in the first dimension on a pH 3–6, 11 cm IPG strip. The numbered spots were prepared for MS analysis and the combined data are presented in Table 1 as gel # 6.

by PMF, 297 (or 78.4%) were identified by MS/MS. Of those, only 84 (28%) were positively identified as *Sus scrofa* proteins, again indicating a lack of overall annotated pig sequences in the public domain. For both PMF and MS/MS analyses, the remainder of the positive identifications were primarily based on bovine, human and mouse sequences. Twenty-four protein spots identified by MS/MS were characterized by "Blasting" a significant swine-EST identification against (non-pig) annotated proteins in either the NCBInr or SwissProt databases. Of those spots identified by an EST search, 11 represented unique protein identifications (3,2-*trans*-enoyl-CoA-isomerase-peroxisomal PECI and mitchondrial, bile acid CoA-amino acid *N*-acyltransferase, dehydrogenase/reductase-SDR1, ester hydrolase, glycine-*N*-acyltransferase, hydroxysteroid dehydrogenase, ketohexokinase, nicotinate-nucleotide pyrophosphorylase, propanoyl-CoA C-acyltransferase and *trans*-2-enoyl-CoA reductase (see Table 1 and Supplement 1). Despite the distinct paucity of available annotated pig sequence data, our observation of 282 unique proteins compares favorably to a similar study where 273 proteins were identified for rat liver where multiple narrow and broad-range 2D-PAGE were performed with very high amounts of cytosol and total protein [19]. In addition, the multiple pH range approach to mapping mammalian tissue proteome appears well suited by comparison to a single large gel format broad-range analysis where 111 proteins were identified from 1000 candidate spots of a human pituitary adenoma [22] or 212 proteins from human kidney glomerulus [23] using similar PMF and MS/MS identification techniques.

In the present study, we have utilized analytical quantities of protein to ensure good separation for future comparative purposes and to provide a detailed 2D map such that the vast majority of protein spots were composed of single proteins. Of the 349 proteins identified by PMF 98.3% were single protein spots and 6 spots were determined to contain two different proteins. With the proteins identified by MS/MS, 90.2% were single protein identifications. Most of the 29 multiple spots were identified with two proteins (23), while 1 spot (gel 1, spot 238) contained 6 unique proteins and another spot (gel 1, spot 239) contained 5 proteins. Of the unique proteins that were identified 130 were limited to the cytosol gels (1, 3 and 5) and were represented by prototypical cytoplasmic proteins such as beta actin, esterase-D, ferritin and lactate dehydrogenase. Eighty-four proteins were observed only in the membrane gels (2, 4 and 6) and these were represented primarily by mitochondrial and peroxisomal proteins including aconitase, succinate dehydrogenase, beta hydroxybutyrate dehydrogenase, ketoacyl thiolase, hydroxyacid oxidase, acylcoenzyme A synthetase. Transferrin (a soluble, secreted protein) was observed in the membrane fraction and probably reflects its association with the lysosome/endosome compartment. The remaining proteins (68) were distributed among all gels and probably represent incomplete initial fractionation and the use of frozen cells as starting material. In addition many gene products may indeed be present in multiple locations within the cell.

Proteins observed were widely distributed among the known functional categories associated with liver metabolism (Fig. 7). Protein metabolic pathways including synthesis, degradation, folding and post-translational modification accounted for the highest percentage of observed proteins (20.2%), followed by lipid metabolism including fatty acid synthesis and oxidation and cholesterol, steroid and bile acid metabolism (18.8%). Cellular energetics, including electron transport, glycolysis, gluconeogeneis, carbohydrate metabolism, and TCA cycle were associated with 14.9% of identified proteins, while amino acid metabolism represented 13.8% and detoxification activities including antioxidant proteins represented 9.2%. As expected, there was a distinct lack of microsomal proteins such as the drug/steroid metabolizing major cytochrome P-450 proteins, in both the membrane and cytosol fractions [24]. Accordingly, proteomic analysis of prepared porcine hepatocyte microsomes, for the specific identification of cytochrome P450 content and activity are currently under investigation in our laboratory.

In conclusion, we have presented the first comprehensive proteomic analysis of porcine hepatic cells (by 2D-PAGE/MS analysis) and in particular, these are the only data that have been assembled identifying major proteins in freshly isolated pig hepatocytes. While porcine hepatic tissue has been previously separated by 2D-PAGE [25,26] to date, only limited protein identification or analyses have been reported. The data presented here will serve as a basis to compare more-refined subcellular fractionation techniques and will allow future comparison of changes associated with cell culture or in vivo endocrine and nutritional interactions.

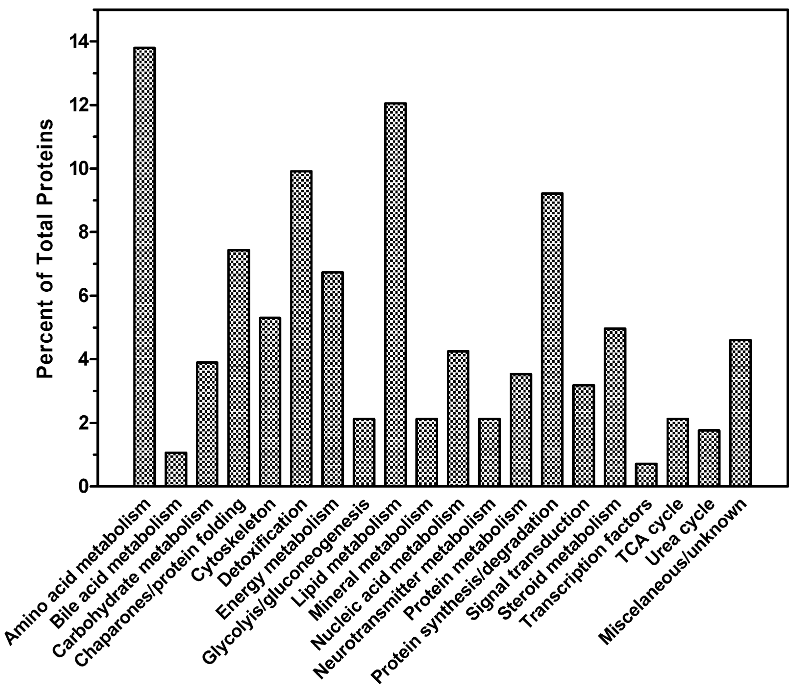

Fig. 7. Classification of uniquely identified proteins from porcine hepatocytes. Main biological functions were grouped according to public database classification (http://www.geneontology.org and http://us.expasy.org/sprot).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.domaniend.2007.12.004.

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